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Research Collaborations Can Improve the Use of Organic Amendments for Plant-Parasitic Nematode Management

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Abstract: The concept of utilizing organic amendments to manage plant-parasitic nematodes is not new, but the widespread implementation of this management practice has still not been realized. The use of organic amendments for plant-parasitic nematode management is a complex process requiring an understanding of the transformation and generation of active compounds. As a result, research endeavors to understand and maximize the use of this management practice require a multidisciplinary approach which draws upon the expertise of nematologists, microbiologists, natural product chemists and soil scientists. Factors that require analysis and clarification include lethal concentration levels of organic amendments necessary to kill nematodes; chemical composition of incorporation material; fate and exposure potential to nematodes of compounds released into the soil; and the influence of environmental factors (i.e., temperature, microbial community, soil type) on the activity of organic amendments. Examples of research conducted in a collaborative manner with rye (Secale cereale) and a biosolid amendment demonstrate the power of multidisciplinary research. Only through collaborative research can consistent and reliable nematode suppression with organic amendments be achieved.

Use of organic amendments to manage plant-parasitic nematodes is not a new concept, but the implementation of this management practice has still not been widely realized. Incorporating organic amendments in a plant-parasitic nematode management program is a complex process that requires knowledge of the generation and transformation of active compounds. As a result, research towards understanding this management practice and maximizing its use requires a multidisciplinary approach. Such an approach will draw upon the expertise of nematologists, microbiologists, natural product chemists, and soil scientists. Factors that require analysis and clarification include determination of concentration levels of organic amendments lethal to nematodes and the chemical composition of incorporation material, the fate of compounds released into the soil and consequent exposure to nematodes, and the influence of environmental factors including temperature, microbial community, and soil type on the activity of organic amendments. The power of multidisciplinary research is clearly illustrated through examples of collaborative research conducted with a biosolid amendment and with the cover crop rye (*Secale cereale*).

Biosolid amendment: Biosolids are the nutrient-rich, solid organic material recovered from treatment of domestic sewage in wastewater treatment facilities. Technologies developed for the treatment of biosolids yield a pathogen-free product that is stable during storage and transportation. One such technology is the mixing of biosolids with alkaline reagents, including industrial by-products (Logan and Burnham, 1995). The final products are solid, granular materials with many desirable agronomic properties. The use of alkaline-stabilized biosolids (ASB) for plant-parasitic nematode management is highly desirable because two undesirable waste products (biosolids and industrial by-products) would be consumed in a positive way. With this goal in mind, a multidisciplinary, multi-institutional research effort was undertaken to evaluate ASB for plant-parasitic nematode management.

Results of research conducted in six states using ASB to control root-knot nematode (*Meloidogyne* spp.) and the soybean cyst nematode (*Heterodera glycines*) shared one common feature, inconsistency (Zasada et al., 2008). This is not surprising considering the diverse environments into which the amendment was added, and the different application rates and methods used. One mechanism by which ASB suppresses nematodes is through the production of gaseous and nematicidal ammonia (NH_3) at elevated soil pH (Meyer et al., 2005; Zasada and Tenuta, 2004; Zasada, 2005). Identifying environments and management practices where this mechanism can be used is essential not only from the perspective of maximizing plant-parasitic nematode suppression, but also from the position of environmental and economic requirements. Elucidating the complex changes that occur in soil chemistry after the addition of ASB to soil requires collaboration with soil scientists.

In collaboration with Mario Tenuta (University of Manitoba), experiments and methodologies were designed to determine how several environmental factors and management practices influenced soil pH/NH_3 dynamics and plant-parasitic nematode suppression after the application to soil of an ASB amendment. In all experiments, ASB was added to *M. incognita*-inoculated soil 5 days prior to planting of a nematode-susceptible plant. The pH and NH_3 concentrations in soil solution were measured at 0, 3 and 5 days, and

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nematode reproduction was determined after approximately 45 days (Meyer et al., 2005; Zasada and Tenuta, 2008). Manipulation of soil temperature and moisture of a loamy sand soil after the addition of ASB had a profound influence on pH/NH_3 dynamics. Maximum and cumulative NH_3 in soil solution was highest at the lowest soil moisture (25% of water holding capacity) and highest temperature (31° C). In addition, simulated tarping (closed bag incubation) at a temperature of 26° C doubled the amount of NH_3 to which nematodes were exposed. The pH of soil solution was not influenced by moisture, temperature, or tarping. We also achieved root-knot nematode (*M. incognita*) suppression at a reduced (2%) ASB rate when it was combined with an additional nitrogen source, urea.

While the use of nitrogenous fertilizers and amendments has received some attention (Rodríguez-Kabána, 1986; Walker, 1971), its adoption as a consistent and reliable pest management practice has not occurred because of incomplete and site-specific nature of disease control, large application rates, and the instability of ammonia in soil (Oka et al., 2006). Through a deeper understanding of changes in soil chemistry after the application of nitrogenous amendments these concerns may be alleviated. Our research provides evidence that ASB should be applied in a site-specific manner and in a managed environment to minimize application rates and maximize efficacy. A resulting example of a successful scenario is the application of ASB to a sandy soil covered with a tarp in a warm environment.

Rye (Secale cereale) cover crop: The winter annual cover crop rye (*Secale cereale*) has been used in cropping systems to reduce soil erosion, recycle nutrients, enhance soil tilth, and reduce inputs of chemical fertilizers and pesticides. Rye also produces secondary plant metabolites (benzoxazinoids) that suppress weeds and other pests (Friebe, 2001; Putnam, 1983). Benzoxazinoids occur as glucosides in intact rye. Upon tissue disruption β-glucosidase is released and the glucosides are rapidly hydrolyzed to release 2,4-dihydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA) and 2,4-hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIMBOA), which subsequently decompose in water to form benxozazolin-2(3*H*)-one (BOA) and 6-methoxy-benzoxazolin-2(3*H*)-one (MBOA), respectively. All of these compounds have been ascribed a role in plant defense, and we have quantified their toxicity to *M. incognita* and *Xiphinema americanum* in vitro (Zasada et al., 2005).

In vitro toxicity studies provide a valuable starting point for assessing the potential of a plant material or amendment to kill nematodes. However, they only provide a guide for subsequent studies because of the complex interactions of amendment-derived compounds with the soil environment. Even if nematotoxic concentrations of benzoxazinoids are contained in rye incorporation material, the fate and nematode exposure potential of these compounds in soil is not yet fully understood. Towards understanding the exposure potentials to *M. incognita* of DIBOA and its degradation product BOA in soil a collaboration with an environmental chemist (Clifford Rice, USDA/ARS) was forged.

When pure DIBOA was added to loam (sand:silt:clay 33:49:18%; 2.9% organic matter (OM)) or loamy sand (sand:silt:clay 64:21:15%; 1.5% OM) soils, and then extracted from soil solution and solid phase over a 24-hour period, there was a difference in the availability of DIBOA between the two soils (Zasada et al., unpublished data). Of the DIBOA recovered after 4 hrs, approximately 70% had adhered to the solid phase in the loam soil compared to 50% in the loamy sand soil. This trend continued over the 24-hour period, with over 80% of the DIBOA being associated with the soil solid phase and less than 20% being found in solution in the loam soil. Nematodes exist in the water-filled spaces between soil particles; therefore, it is likely they are exposed only to compounds in soil solution, not those adhering to the solid phase (i.e., clay particles, organic matter). Our results are significant because they demonstrate that the level of exposure of nematodes to DIBOA is potentially 20% greater in a soil of loamy sand compared to a loam soil.

In a greenhouse experiment, we added pure DIBOA to a loamy sand soil inoculated with *M. incognita*. DIBOA was applied at a range of concentrations including ones that corresponded to the concentration necessary to kill 50% (LD_{50}) of an *M. incognita* egg population (Zasada et al., 2005), up to 2X LD_{50}. DIBOA concentration in soil was monitored over a five-day period and nematode reproduction assessed (eggs/g root) after a month. A rate of twice the LD_{50} of DIBOA resulted in a non-significant decrease in *M. incognita* survival, with lesser concentrations of DIBOA having little affect (Meyer et al., 2009). This was not surprising when the DIBOA concentration in soil over time data was evaluated. In a loamy sand soil the half-life of DIBOA was < 24 hrs. It is likely that nematodes were exposed to the maximum concentration necessary to cause mortality for only a very short period of time.

Examples from research striving to improve the use of rye cover crops and biosolid amendments to manage plant-parasitic nematodes highlight the need for a deeper understanding of the factors influencing amendment efficacy pre- and post-incorporation into soil. To start, only amendment materials which have the potential to generate of nematotoxic concentrations should be selected and used. These materials should be recommended for plant-parasitic nematode management only under environmental and soil conditions which have been experimentally qualified by suppression studies. Our research clearly shows that both ASB and a rye cover crop will more effectively suppress plant-parasitic nematodes in sandy soils. Interdiscplinary research allows nematologists to ask more complex questions and utilize technologies otherwise unavailable, to address what happens to amendments once they are incorporated into soil and how the effects of these incorporated amendments can be maximized in order to obtain consistent and reliable nematode suppression.

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